Reference: *Biol. Bull.*, 153: 228–236. (August, 1977)

METABOLIC RELATIONSHIPS BETWEEN GREEN HYDRA AND ITS SYMBIOTIC ALGAE

ROSEVELT L. PARDY[1] AND BRIAN N. WHITE[2]

Department of Developmental and Cell Biology, University of California, Irvine, California 92717

Green hydra are characterized by the presence of symbiotic, green algae in their digestive cells. These *Chlorella*-like (= zoochlorellae) symbionts have been shown to be photosynthetically active (Lenhoff and Muscatine, 1963) to facilitate the survival of the host under starvation conditions (Muscatine and Lenhoff, 1965) and to release carbohydrate (Muscatine, 1965). Symbiotic algae that have been isolated from the host and examined *in vitro* at low pH, release up to 85% of their photosynthetically-fixed ^{14}C as maltose (Muscatine, 1965). While the aforementioned studies show that algal symbionts translocate products to the host and that large quantites of carbohydrates are potentially available, no measurements have been made reporting how much material is actually translocated and utilized in the intact association. In this work the metabolic requirements of green hydra under various conditions are estimated, and the major class of metabolites being burned are deduced. Moreover, the caloric demands of the association are compared with the calories potentially available from the nutrition provided by exogenous food and available from the symbiotic algae.

The analysis consisted of measuring the respiration rates of green hydra maintained under various feeding and light regimes. From the respiration rates, respiratory quotients were calcaulated and compared. The respiratory quotient (RQ) is defined as the ratio of carbon dioxide produced to oxygen consumed during respiration (Kleiber, 1965). This ratio, which varies from 0.7 to 1.0 is indicative of the kind of food being oxidized (fat or carbohydrate) and can be used to calculate the caloric expenditure of an organism and the percentage of energy derived from carbohydrate and fat catabolism. The theoretical basis of respiratory quotients and caloric estimation (called indirect calorimetry) is a standard topic in most physiology texts (see Wilson, 1972) and is discussed at length by Kleiber (1961). The results of these experiments were compared to determinations made using aposymbiotic (algae-free) hydra.

Materials and Methods

Experimental animals

Green and aposymbiotic (= algae-free) hydra were grown and maintained in M solution according to the methods of Lenhoff and Brown (1970). The animals were originally a gift of Dr. L. Muscatine and, lacking specific taxonomic designation, are referred to as the English strain of green hydra. These animals are dis-

[1] Present address: Department of Life Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68509.

[2] Present address: Department of Biological Sciences, University of Southern California, os Angeles, California 90007.

tinguished from other green hydra in this laboratory by their larger size, and nematocyst dimensions (Pardy, 1976a). Aposymbiotic hydra were obtained from mass culture of algae-free clones prepared originally by bleaching green animals (Pardy, 1976b). In practice, adult green animals were exposed continuously to light (620 watts/m^2) provided by a General Electric reflector flood lamp while in the presence of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea, K and K Laboratories, Plainview, N.Y.] at 10^{-6} M prepared in M solution. During this treatment the animals were maintained unfed at $15 \pm 1°$ C. Bleached animals resulting from this procedure were reared through several algae-free generations prior to their use in experiments. All experimental cultures were maintained in a photoperiod incubator at $18 \pm 1°$ C with a 12-hour light/dark cycle. Illumination was provided by six 20-watt, cool-white fluorescent tubes. In some instances animals were maintained in the dark for five days prior to experimentation.

Manometry

Gas uptake by hydra was determined using a Roger Gilmont, differential microcapillary manometer as described by Peterson, Freund, and Gilmont (1967). Uptake measurements were made at 20° C by partial immersion of the manometric apparatus in a constant temperature water bath. In practice 0.2 ml of wet packed hydra (130–200 animals, Pardy and Dieckmann, 1975), adjusted to a volume of 2 ml with M solution, were transferred to a Warburg reaction vessel. In experiments measuring total oxygen uptake, twelve drops of 10% KOH were added to the center well of the Warburg flasks to absorb the CO_2 evolved by the respiring hydra. The center wells were provided with filter paper wicks to facilitate rapid CO_2 absorption. Following a 30–45 minute equilibration period, readings of oxygen uptake were taken every five minutes for periods up to 90 minutes. The number of microliters of oxygen taken up in each of four or six flasks was regressed against time using the method of least squares. From the slopes of the regression lines the rate of oxygen uptake was calculated. Following correction of the rates for the protein content of the flasks (described below) the average respiration rate per milligram of protein per hour was computed [$= \mu l\ O_2/(mg \cdot hr)$] for the flasks. To avoid the complications of algal photosynthesis and concomitant release of oxygen in green hydra (Pardy and Dieckmann, 1975), all manometric measurements were made in the dark, provided by wrapping the reaction vessels with aluminum foil.

Oxygen uptake measurements were made on green hydra that were fed every 24 hours or unfed (= fasted). These animals were either maintained under a 12L : 12D photoperiod or kept in constant darkness. Aposymbiotic animals were fed every 24 hours or fasted and maintained under a 12L : 12D photoperiod. In all cases, the animals were maintained on an experimental regimen at least five days prior to experimentation and were fasted 24 hours before respiration measurements were made.

Protein determination

At the end of each experiment, the animals were carefully removed from each flask and analyzed for protein content by the method of Lowry, Rosebrough, Farr and Randall (1951). Animals were homogenized using a tissue grinder and

motorized pestle. Symbiotic algae, which are unaffected by homogenization, were separated from the host tissue by low speed centrifugation ($50 \times g$). Protein analyses were then performed on the algae-free host homogenate.

Determination of respiratory quotient (RQ)

The respiratory quotient was determined experimentally by measuring the rates of oxygen uptake in the presence of the CO_2 absorbant (10% KOH placed in the center wells) and, in the absence of the CO_2 absorbant, according to the methods discussed by Umbriet, Burris and Stauffer (1964). After determining the oxygen consumption rate the animals were removed from the reaction vessels and resuspended in fresh M solution in flasks with no KOH in the center wells. Following a 45 min equilibration period, readings were taken at five min intervals for periods of up to one hour. The rate of carbon dioxide given off during respiration was taken to be the difference between the rate of oxygen uptake measured in the presence of KOH and that measured with no KOH. In these experiments RQ = (rate of CO_2 evolution)/rate of O_2 consumption) = (rate of O_2 consumption + KOH) − (rate of O_2 consumption − KOH)/(rate of O_2 consumption + KOH). The caloric equivalence of these experimentally determined RQs was determined by comparing them to the values given in standard tables by Richardson (1929).

Growth rates

In these experiments respiration rates were measured as a function of various feeding/light conditions. To assess the growth of the animals under these regimes, replicate groups of animals were maintained in 9 cm plastic petri dishes under identical conditions as the experimental hydra. Daily for seven days, the number of hydranths in each dish were counted and the growth rates and population doubling times were calculated using the method of Loomis and Lenhoff (1956).

Results

When RQ values are determined for higher animals, such as vertebrates, respiration rates are corrected for protein catabolism by measuring the amount of urea or uric acid nitrogen excreted during an experimental period. The degree of protein catabolism in hydra was estimated by measuring the decrease in protein in populations of aposymbiotic hydra over a period of eleven days during which the animals were not fed. Starting with populations that had 1.3 mg of protein on day 1, a decrease of 0.5 mg protein (determined as discussed under Materials and Methods) at day 11 was detected. The catabolism of this amount of protein would require approximately 1.24×10^{-2} μl of oxygen per mg protein per day if the measured decrease in protein was due to catabolic losses alone and not due to tissue sloughing as described by Campbell (1967, 1974). Under the conditions of these experiments, this amount was judged to be insignificant and corrections of the respiration data for protein catabolism were not made.

The rates of oxygen uptake of the hydra under various conditions are shown in Table I, as are the growth rates and doubling times of the animals. The green

TABLE 1

Rates of oxygen uptake and CO_2 evolution by green and aposymbiotic hydra as determined with and without absorption of respired CO_2 (+ or −KOH) under various conditions. Data are expressed as mean ± the standard deviation and $n = 4$ for all except fed light where $n = 6$. Growth rate constant (K) and population doubling time in days (d.t.) is given for each experimental group of animals.

Hydra and experimental conditions	Oxygen uptake μl O_2/(mg·hr)		CO_2 given off μl CO_2/(mg·hr)
	+KOH	−KOH	
Green hydra			
Fed light (K = 0.187, d.t. = 3.7)	55.2 ± 13.7	7.7 ± 2.4	47.5
Fed dark (K = 0.187, d.t. = 3.7)	20.5 ± 9.9	6.3 ± 6.0	14.2
Fasted light (K = 0.020, d.t. = 34.6)	21.2 ± 7.2	1.7 ± 1.6	19.5
Fasted dark (K = 0, d.t. = 0)	17.5 ± 5.6	5.1 ± 3.5	12.4
Aposymbiotic hydra			
Fed (K = 0.17, d.t. = 3.9)	17.5 ± 2.8	5.4 ± 1.7	12.1
Fasted (K = 0.03, d.t. = 21.6)	18.7 ± 2.7	4.1 ± 1.7	14.6

animals maintained under a 24 hour feeding regimen with light exhibited an uptake rate of 55.2 ± 13.7 μl O_2/(mg·hr). This rate was over twice that of animals, aposymbiotic or green, maintained under other experimental conditions, although the growth of these animals did not appear significantly different from the other fed animals (Table I). An analysis of variance showed that the rates of oxygen uptake for the green hydra (fed-dark, fasted-light, fasted-dark) and aposymbiotic hydra (fed or starved) were not significantly different ($F = 0.300$, $P > 0.75$). When the rate for the fed-light experiment (green animals) was included in the analysis, $F = 13$ and $P < 0.001$. In subsequent calculations the rate of oxygen uptake was taken to be the average of all the rates [19.1 μl O_2/(mg·hr)] excepting that determined for the green animals maintained on the fed-light regimen.

An analysis of variance performed on rates of oxygen uptake without KOH by hydra maintained under various conditions (Table I) showed no significant difference ($F = 0.616$, $P > 0.50$) between green hydra (fed-light, fed-dark, fasted-dark) or aposymbiotic hydra (fed, fasted). When the values for the fasted-light experiments were included, $F = 1.584$, $0.25 > P > 0.10$. In further calculations the rate of oxygen uptake in the absence of KOH was taken to be the average of the experiments above [5.5 μl O_2/(mg·hr)] exclusive of the rate for the green, fasted-light animals. The amount of CO_2 given off during the various experiments is shown in Table I. Green hydra maintained under a 24 hour feeding regimen with light exhibited a rate of CO_2 evolution over twice that determined for the other experiments.

Lenhoff (1965) determined the protein content for single specimens of *Hydra viridis* to be 6.32 μg. Using this factor and assuming that the animals used in the

TABLE II

Comparison of oxygen consumption, RQ, energy expenditure and amounts of fat and carbohydrate consumed by hydra under various experimental conditions.

Hydra and experimental conditions	Oxygen consumption μl/day per hydra	RQ	Number and percent of calories expended per day		Total calories per day	Estimated amount of food (μg) catabolized per hydra per day	
			Fat	Carbohydrate		Fat	Carbohydrate
Green hydra Fed-dark Fasted-dark Aposymbiotic hydra Fed Fasted	5.7	0.725	0.0305 (95.2%)	0.0015 (4.8%)	0.0320	3.0	3.7
Green hydra Fed-light	14.4	0.862	0.0320 (45.9%)	0.0302 (54.1%)	0.0702	3.0	9.5
Fasted-light	5.7	0.970	0.0072 (25.9%)	0.0208 (74.1%)	0.0280	0.8	5.2

present experiments were about twice the size of *H. viridis* (based on length measurements), it was estimated that their protein content was about 12.6 μg per hydra. This value was used in converting the data in Table I, which are expressed as μl O_2/mg protein·hr, to μl O_2/hydra·hr. Since the hydra are fed only once in 24 hours and growth rates and doubling times are generally expressed in days, the data were converted to μl O_2/hydra·day in constructing Table II. From Table II, it can be seen that green hydra maintained under fed-light conditions exhibited an RQ of 0.862 *vs.* 0.920 for green animals fasted with light. The other experimental conditions and animals yielded an RQ of 0.725. Using these data and values presented in standard tables (Richardson, 1929) the calories expended per day per hydra and the amount of calories derived from fat and carbohydrate catabolism were calculated. Finally, from the caloric values, the numbers of grams of fat and carbohydrate burned per day were calculated using the relationship of 9.5 kilocalories per gram of fat and 4.0 kilocalories per gram of carbohydrate (Kleiber, 1965). The results of these calculations are shown in Table II from which the following points emerge. First, fed green hydra maintained in the light consume over twice the amount of oxygen and expend over twice the amount of energy as the animals in the other experiments and by comparison, catabolize a relatively large amount of carbohydrate. These animals catabolize approximately the same amount of fat (3.3 μg) as the other experimental animals with the exception of green hydra starved in the light (0.8 μg). Secondly, green hydra starved with light expend approximately the same amount of energy (0.0280 cal/day) as the green hydra maintained in the dark (fed or fasted) and the aposymbiotic animals (0.0320 cal/day). By contrast the green hydra fasted in the light appear to derive 74% (5.2 μg) of their energy from carbohydrate *vs.* 4.8% (3.7 μg) for the other animals.

Discussion

Values for the respiratory quotient vary typically from 0.7, for fat catabolism, to 1.0 for pure carbohydrate catabolism with intermediate values indicating catabolism of mixed amounts of fat and carbohydrate (Kleiber, 1965). In these experiments three different RQ values were obtained which varied according to the regimen under which the hydra were maintained. An RQ of 0.725 (Table II) was determined for fed or fasted aposymbiotic animals and for green animals maintained in the dark. Under these conditions, the hydra expended energy at the rate of 0.032 calories per day.

According to Slobodkin and Richman (1961) an individual *Artemia* nauplius weighs about 1.48 μg and has a caloric content of 0.0091 calories. Urbani (1959) measured the amount of fat and carbohydrate in *Artemia* nauplii and reported values of 0.22 μg per nauplius for fat and 0.347 μg per nauplius for carbohydrate. These amounts correspond to 0.0021 calories per nauplius for fat and 0.0013 calories per nauplius for carbohydrate. From Table II it can be seen that a hydra which exhibits an RQ of 0.725 and which consumes 5.7 μl O_2/day requires at least 3.0 μg of fat and 3.7 μg of carbohydrate. Hydra fed in these experiments ate between 8 and 12 nauplii per hydra per day and hence ingested 1.77 μg to 2.66 μg of fat (representing 0.0168 to 0.0252 calories) and 2.77 μg to 4.16 μg of carbohydrate (representing 0.0118 to 0.166 calories). The assimilation efficiency of the carbohydrate and fat components of ingested brine shrimp nauplii are not known, hence estimates are necessarily provisional. However, it is clear from these calculations that the amount of fat and carbohydrate required by fed hydra as deduced from our metabolic data and that which is potentially available from *Artemia* are in fairly good agreement. Fed animals, however, appear not to catabolize fat and carbohydrate in proportion to the amounts present in their food but seem to oxidize fat preferentially. The ratio of carbohydrate to fat in a nauplius is about 1.56, whereas the ratio of carbohydrate to fat metabolized by the hydra is approximately 0.12. Moreover, it is obvious that fed animals obtain more carbohydrate from their food than necessary to account for the observed RQ of 0.82. It is possible that this excess carbohydrate is not catabolized but rather is stockpiled as glycogen or utilized in the synthesis of other molecules without undergoing oxidation.

Fat metabolism was also indicated in fasted hydra—a condition which is obtained in higher animals (Kleiber, 1965). Kleiber (1965) reports that the caloric expenditure for fasting animals is taken to be 4.7 kcal per liter oxygen consumed. Using this factor, a value of 0.0267 calories per hydra per day is obtained which approximates the values (0.0320 calories) that were derived from the respiratory data.

Fed green hydra maintained in the light exhibited a higher respiratory rate (Tables I and II) than animals under any other condition. These animals exhibited an RQ of 0.862, indicating a mixed metabolism with a proportionate increase in carbohydrate catabolism. The source of this carbohydrate must be the algal symbionts for two reasons. First, significant carbohydrate metabolism is observed only in green hydra maintained in the light (Table II). Secondly, a maximum of 4.06 μg of carbohydrate is potentially available from hydra's food *vs.* the 9.5 μg

predicted necessary for the estimated RQ of 0.862 at the oxygen consumption of 14.4 μl/day per hydra exhibited by these fed animals. The increased oxygen consumption measured in these hydra (Tables I, II) probably results from the catabolism of carbohydrates by the symbionts. Pardy (1974) has shown that green hydra symbionts do not reproduce when the host is starved with or without light. Conversely, symbiotic algae increase logarithmically, as do the hosts when the hydra are fed and maintained in the light (Pardy, 1974). The animals in the fed-light experiment were from populations growing logarithmically at a rate constant of 0.18 (Table I). Hence it is hypothesized that the observed enhanced oxygen consumption and caloric expenditure is most likely a function of the increased metabolic and biosynthetic activity associated with symbiont reproduction. Table I shows that the animal constituent of the symbiosis under these growing conditions requires the same amount of fat as predicted for the aposymbiotic hydra and green hydra maintained in the dark (3 μg), a quantity potentially available in the animal's diet of shrimp nauplii.

Fasted green hydra maintained in the light exhibit an RQ of 0.970 and an oxygen consumption rate of 5.7 μl/day. These animals show a very low growth rate constant (Table I). It is under these conditions that symbiotic hydra demonstrate enhanced survival when compared with aposymbiotic hydra (Muscatine and Lenhoff, 1965). The RQ of 0.97 derived from these data results from a decrease in the amount of fat catabolized with a concomittant increase in carbohydrate oxidation. The caloric expenditure of 0.0280 is within 88% of the estimate for fed animals and is derived from the catabolism of at least 5.2 μg of carbohydrate. The source of this carbohydrate, in view of the fact that the animals were receiving no exogenous food, must be the photosynthetic symbionts. The algae, under conditions of light and host starvation, do not reproduce (Pardy, 1973) but are photosynthetically active. The nutrients produced by the algae are shunted to the animal partner, where they are catabolized rather than being converted into new algal biomass. This process apparently spares the animal of its fat reserves (fat metabolism is reduced 75%) and thus the survival of the animal, and hence the system, is prolonged.

One process that was not accounted for in these experiments was the photorespiration of the algae. Many plants show a marked stimulation of nonmitochondrial respiration in the light. The measurement of this respiration is difficult as the substrates and products (CO_2, O_2) are common to the photosynthetic pathway. This difficulty was circumvented to a degree by performing respiratory measurements in the dark. A detailed discussion of photorespiration is found in Zelitch (1971) who points out that free-living *Chlorella* exhibit measurable photorespiration only under conditions of abnormally high oxygen concentrations or abnormally low carbon dioxide concentration. As little is known about the intracellular concentration of these substrates in hydra, estimates of photorespiration are not possible at this time.

These experiments support the generally held notion that symbiotic algae provide nutrition to hydra when the animals are undergoing starvation. In contrast to previous workers, the present efforts have yielded some reasonable estimates about the quantities of foodstuffs and energy involved. We offer the RQ of 0.970 obtained under host fasting in the light as evidence that the algae supply

carbohydrate to the host and that at a respiration rate of 5.7 μl/day at least 69% of the animal's energy requirements may be supplied by the symbionts.

From this work at least two predictions emerge to be tested. First, the algae in a single hydra must be capable of releasing or translocating at least 5.2 μg of carbohydrate. Secondly, algae from hydra fed and growing in the light direct most of their photosynthate to the biosynthesis of new algae material whereas algae from unfed hydra maintained in the light divert most of their photosynthate to the host. Hence algae from starving hydra should be significantly "more leaky" than algae from fed animals.

From these respiratory measurements, it is evident that under most conditions hydra (green or aposymbiotic) obtain the major proportion of their energy from fat. When green hydra undergo starvation, the symbionts provide nutrition and the animals switch from a fat to a carbohydrate metabolism. It seems reasonable to expect that as the algae become more prone to translocation, biochemical or enzymatic switching must take place within the metabolic network of the animal cells. How the host and symbiont metabolisms are modulated is not known but control may involve host feeding. When a hydra is fed, both the animals and algae (if light is present) reproduce; when the animals are starved, reproduction of both partners ceases. Starvation conditions in the host may signal cessation of algal reproduction with concomitant diversion of carbohydrate to the host. Current work in this laboratory is directed toward examining some of these possibilities.

This research was supported by ASPHS Grant RRO7008.

Summary

1. Hydra that maintain algal symbionts evidenced three modes of metabolism depending upon the nutritional state of the host and the photoperiodic conditions under which the animals were maintained. Animals either fed or fasted but maintained in the dark exhibited an RQ of 0.725 indicating fat metabolism. When they were fed *Artemia* nauplii and maintained in the light, green hydra exhibited a mixed metabolism of carbohydrate and fat which gave an RQ of 0.862. Fasting green hydra, when maintained in the light, showed a pronounced carbohydrate metabolism typified by an RQ of 0.970.

2. Aposymbiotic hydra, whether fed or fasted, exhibited an RQ of 0.725 indicating a high degree of fat metabolism.

3. Symbiotic hydra which were fed and maintained in the light demonstrated a respiration rate of 14.4 μl/day per hydra, which was 2.5 times greater than animals (symbiotic or aposymbiotic) maintained under any other condition of fasting or photoperiod. It is hypothesized that the enhanced respiration observed in these animals is due to the metabolic activities of the symbiotic algae associated with reproduction and development.

4. Calculations based on respiratory measurements and indirect calorimetry suggest that hydra consume approximately 0.0320 calories per day and that symbiotic algae may supply up to 69% of the host caloric requirements *via* the translocation of approximately 5 μg of carbohydrate when the host is fasting and light is provided.

LITERATURE CITED

CAMPBELL, R. D., 1967. Tissue dynamics of steady state growth in *Hydra littoralis*. II. Patterns of tissue movement. *J. Morphol.*, **121**: 19–28.

CAMPBELL, R. D., 1974. Cell movements in *Hydra*. *Am. Zool.*, **14**: 523–535.

KLEIBER, M., 1961. *Fire of life*. John Wiley and Sons, New York, 454 pp.

KLEIBER, M., 1965. Respiratory exchange and metabolic rate. Pages 927–938 in W. O. Fenn and H. Rahn, Eds., *Respiration, Volume II*. American Physiological Society, Washington, D.C.

LENHOFF, H. M., 1965. Cellular segregation and heterocytic dominance in hydra. *Science*, **148**: 1105–1107.

LENHOFF, H. M., AND R. BROWN, 1970. Mass culture of hydra: an improved method and its application to other aquatic invertebrates. *Laboratory Animals*, **4**: 139–154.

LENHOFF, H. M., AND L. MUSCATINE, 1963. Symbosis: on the role of algae symbiotic with hydra. *Science*, **142**: 956–958.

LOOMIS, W. F., AND H. M. LENHOFF, 1956. Growth and differentiation of hydra in mass culture. *J. Exp. Zool.*, **132**: 555–573.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL, 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**: 267–275.

MUSCATINE, L., 1965. Symbiosis of hydra and algae. III. Extracellular products of the algae. *Comp. Biochem. Physiol.*, **16**: 77–92.

MUSCATINE, L., AND H. M. LENHOFF, 1965. Symbiosis of hydra and algae. II. Effects of limited food and starvation on growth of symbiotic and aposymbiotic hydra. *Biol. Bull.*, **129**: 316–328.

PARDY, R. L., 1973. Regulation of the endosymbiotic algae in hydra by digestive cells and tissue growth. *Am. Zool.*, **14**: 583–588.

PARDY, R. L., 1974. Some factors affecting the growth and distribution of the algal endosymbionts of *Hydra viridis*. *Biol. Bull.*, **147**: 105–118.

PARDY, R. L., 1976a. The morphology of green hydra endosymbionts as influenced by host strain and host environment. *J. Cell Sci.*, **20**: 654–669.

PARDY, R. L., 1976b. The production of aposymbiotic green hydra by the photodestruction of their symbiotic algae. *Biol. Bull.*, **151**: 225–235.

PARDY, R. L., AND C. L. DIECKMANN, 1975. Oxygen consumption by the green hydra, *Hydra viridis*. *Exp. Zool.*, **194**: 373–378.

PETERSON, R. N., M. FREUND, AND R. GILMONT, 1967. Measurement of low rates of oxygen consumption with a horizontal capillary-differential syringe manometer. *Proc. Soc. Exp. Biol. Med.*, **125**: 645–648.

RICHARDSON, H. B., 1929. Respiratory quotient. *Physiol. Rev.*, **9**: 61–125.

SLOBODKIN, L. B., AND S. RICHMAN, 1961. Calories per gram in species of animals. *Nature*, **191**: 299.

UMBRIET, W. W., R. H. BURRIS, AND J. J. STAUFFER, 1964. *Manometric techniques, 4th ed.* Burgess Publishing Co., Minneapolis, 304 pp.

URBANI, E., 1959. Protidi, glucidi e lipidi nello sviluppo di *Artemia salina* Leach. *Acta. Embryo. Morph. Exp.*, **2**: 171–194.

WILSON, J. A., 1972. Pages 636–637 in *Principles of animal physiology*. Macmillan Co., New York.

ZELITCH, J., 1971. Dark respiration and photorespiration. Pages 130–169 in *Photosynthesis, and plant productivity*. Academic Press, New York, 375 pp.